UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/076,416	02/19/2002	Mechthild Rieping	218162US0X	2415

22850 7590 11/09/2010

OBLON, SPIVAK, MCCLELLAND MAIER & NEUSTADT, L.L.P.
1940 DUKE STREET
ALEXANDRIA, VA 22314

EXAMINER	
STEADMAN, DAVID J	
ART UNIT	PAPER NUMBER
1656	

NOTIFICATION DATE	DELIVERY MODE
11/09/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@oblon.com
oblonpat@oblon.com
jgardner@oblon.com

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/076,416
Filing Date: February 19, 2002
Appellant(s): RIEPING ET AL.

Daniel J. Pereira
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 10/18/10 appealing from the Office action mailed 11/20/09.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The following is a list of claims that are rejected and pending in the application:

Claims 23, 26-28, 33, 35-42 are pending in the application.

Claims 23, 26-28, 33, and 39-42 are rejected.

Claims 35-38 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

The claims on appeal are 23, 26-28, 33, and 39-42.

(4) Status of Amendments After Final

The examiner has no comment on the appellant's statement of the status of amendments after final rejection contained in the brief.

(5) Summary of Claimed Subject Matter

The examiner has no comment on the summary of claimed subject matter contained in the brief.

(6) Grounds of Rejection to be Reviewed on Appeal

The examiner has no comment on the appellant's statement of the grounds of rejection to be reviewed on appeal. Every ground of rejection set forth in the Office action from which the appeal is taken (as modified by any advisory actions) is being maintained by the examiner except for the grounds of rejection (if any) listed under the subheading "WITHDRAWN REJECTIONS." New grounds of rejection (if any) are provided under the subheading "NEW GROUNDS OF REJECTION."

(7) Claims Appendix

The examiner has no comment on the copy of the appealed claims contained in the Appendix to the appellant's brief.

(8) Evidence Relied Upon

5,932,453	KIKUCHI et al.	08-1999
5,445,948	SHIMIZU et al.	08-1995
4,391,907	MATSUI et al.	07-1983
6,586,214	DUNICAN et al.	07-2003

CHANG et al., "Genetic and Biochemical Analyses of Escherichia coli Strains Having a Mutation in the Structural Gene (poxB) for Pyruvate Oxidase", Vol. 154, pp. 756-762, 1983

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

(A) Claims 23, 26, 28, 33, and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kikuchi et al. (US Patent 5,932,453; reference C in the Form PTO-892 mailed on 4/29/09; hereafter "Kikuchi") in view of Shimizu et al. (US Patent 5,445,948; reference B in the Form PTO-892 mailed on 4/29/09; hereafter "Shimizu") and Chang et al. (*J Bacteriol* 154:756-762, 1983; reference V in the Form PTO-892 mailed on 10/19/05; hereafter "Chang").

The reference of Kikuchi teaches methods for producing and collecting L-amino acids, in particular L-lysine and L-threonine, from a culture medium using *Escherichia coli*, including a step of measuring the amount of L-lysine produced (column 9, line 62 to column 10, line 52 and column 15, line 61 to column 18, line 55) and further discloses genetically engineered *E. coli* strains that overproduce L-threonine (column 1, lines 34-49). According to Kikuchi, the culturing time for L-amino acid production is "from 16 to 72 hours" (column 10, lines 44-45). Although not expressly taught by Kikuchi, culturing the *E. coli* of Kikuchi to produce L-lysine or L-threonine results in concentrating the L-lysine or L-threonine in the culture medium as required in claim 23.

The difference between the method of Kikuchi and the claimed method is that the *E. coli* used in the method of Kikuchi does not have an inactivated *poxB* gene.

The reference of Shimizu teaches culturing *E. coli* results in accumulation of acetate (also referred to as "acetic acid") in the culture medium and further teaches that acetate inhibits the growth of *E. coli* in culture (column 4, lines 12-50), and discloses

that removing the acetate from the culture medium enhances production of a desired product produced by *E. coli* (column 6, lines 34-40). Shimizu teaches, "the acetic acid concentration in culture broth should be controlled so as to be…preferably 6 g/liter or less" (column 4, lines 44-47) and noting that "[t]he acetic acid concentration was 6 g/liter at 10 hours of culture".

Shimizu teaches removing acetate by centrifugation (column 7, lines 20-24), rather than by inactivation of *poxB*.

The reference of Chang teaches *E. coli* pyruvate oxidase catalyzes the decarboxylation of pyruvate to form acetate (p. 756, column 1, top) and that the *poxB* gene encodes pyruvate oxidase (p. 762, column 1, top). Chang teaches *E. coli poxB* mutants with an inactivated *poxB* gene, wherein the gene is inactivated by insertion of a transposon into the chromosomal *poxB* gene (see, *e.g.,* p. 758, Table 2 and p. 759, right column). Chang teaches culturing of the *E. coli poxB* mutants (p. 756, column 2) and that the mutants grew at a normal rate on acetate-containing media and attained a normal cell density (p. 758, column 1, middle). According to Chang, growth of *E. coli* on acetate produced by pyruvate oxidase is "feeble" and teaches that preliminary results indicate that most of the endogenous production of acetate is blocked in an *E. coli* strain with an inactivated *poxB* gene (p. 762, column 1, top to middle).

At the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Kikuchi, Shimizu, and Chang for a method of producing an L-amino acid using an *E. coli* with an inactivated *poxB* gene. Although Kikuchi does not expressly teach *E. coli* has a *poxB* gene comprising SEQ ID NO:1

prior to inactivation, this is an inherent feature of the *E. coli poxB* gene, which gene was well-known at the time of the invention as shown by Chang. One would have been motivated to do this in view of the following: the culturing time of Kikuchi (16-72 hours) would result in production of an undesired amount of acetate as supported by Shimizu's results (greater than 6 g/liter); Shimizu and Chang teach acetate inhibits growth of *E. coli* in culture; Chang teaches pyruvate oxidase catalyzes the production of acetate, which is blocked in an *E. coli* with an inactivated *poxB* gene; and Chang teaches the *E. coli poxB* mutants have a normal growth rate and cell density in medium containing acetate. One would have had a reasonable expectation of success for a method of producing an L-amino acid using an *E. coli* with an inactivated *poxB* gene because of the results of Kikuchi, Shimizu, and Chang. Therefore, the method of claims 23, 26, 28, 33, and 42 would have been obvious to one of ordinary skill in the art at the time of the invention.

(B) Claim 27 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kikuchi in view of Shimizu and Chang as applied to claims 23, 26, 28, 33, and 42 above and further in view of Matsui et al. (US Patent 4,391,907; hereafter "Matsui").

The teachings of Kikuchi, Shimizu, and Chang as applied to claims 23, 26, 28, 33, and 42 are set forth above. The difference between the method suggested by the combination of Kikuchi, Shimizu, and Chang and the method of claim 27 is that the combined teachings of Kikuchi, Shimizu, and Chang do not teach or suggest *E. coli* production of L-valine.

However, the reference of Matsui teaches the use of *E. coli* as an L-valine production host (*e.g.,* column 6). The method of Matsui encompasses culturing of *E. coli* for a time of 72 hours (column 4, lines 64-65).

At the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Kikuchi, Shimizu, Chang, and Matsui to inactivate the *poxB* gene in an *E. coli* for use in production and isolation of L-valine. One would have been motivated to inactivate a *poxB* gene in an *E. coli* for use in production of L-valine in view of the following: the culturing time of Matsui (72 hours) would result in production of an undesired amount of acetate as supported by Shimizu's results (greater than 6 g/liter); Shimizu and Chang teach acetate inhibits growth of *E. coli* in culture; Chang teaches pyruvate oxidase catalyzes the production of acetate, which is blocked in an *E. coli* with an inactivated *poxB* gene; and Chang teaches the *E. coli poxB* mutants have a normal growth rate and cell density in acetate medium. One would have been motivated to use an *E. coli* with an inactivated *poxB* in the production of L-valine because Matsui teaches *E. coli* is useful in the production of L-valine. One would have had a reasonable expectation of success to inactivate the *poxB* gene in an *E. coli* for use in production and isolation of L-valine because of the results of Kikuchi, Shimizu, Chang, and Matsui. Therefore, the method of claim 27 would have been obvious to one of ordinary skill in the art at the time of the invention.

(C) Claim(s) 39-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kikuchi in view of Shimizu and Chang as applied to claims 23, 26, 28, 33, and 42 above and further in view of Dunican et al. (US Patent 6,586,214; hereafter "Dunican").

The teachings of the references of Kikuchi, Shimizu, and Chang as applied to claims 23, 26, 28, 33, and 42 are set forth above. The difference between the method suggested by the combination of Kikuchi, Shimizu, and Chang and the method of claims 39-41 is that the combined teachings of Kikuchi, Shimizu, and Chang do not teach or suggest inactivating the *E. coli poxB* gene by deletion mutagenesis (claim 39), homologous recombination (claim 40), and/or transition or transversion mutagenesis (claim 41).

The reference of Dunican teaches methods for attenuating gene expression, including “transitions, insertions, deletions and transversions” and teaches that such methods are achieved by known techniques (column 6, lines 17-61). Dunican goes on to teach attenuation by deletion mutagenesis, homologous recombination, and transition or transversion mutagenesis (*e.g.,* claim 1).

At the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Kikuchi, Shimizu, Chang, and Dunican to inactivate an *E. coli poxB* gene by deletion mutagenesis, homologous recombination, or transition or transversion mutagenesis and use the resulting *E. coli* in the method of Kikuchi. One would have been motivated to do this because Dunican teaches such methods are known for gene inactivation and require only routine techniques to practice. One would have had a reasonable expectation of success to inactivate an *E. coli poxB* gene by

deletion mutagenesis, homologous recombination, or transition or transversion mutagenesis and use the resulting *E. coli* in the method of Kikuchi because of the results of Kikuchi, Shimizu, Chang, and Dunican. Therefore, the method of claims 39-41 would have been obvious to one of ordinary skill in the art at the time of the invention.

(10) Response to Argument

At p. 5 of the Brief, appellant argues the examiner's assertion that Shimizu teaches that acetate generally inhibits growth of *E. coli* in culture is not true. According to appellant, Shimizu teaches a concentration above 17 g/L of acetate inhibits the growth of *E. coli*.

Appellant's argument is not found persuasive. Contrary to appellant's assertion, the rejection does not state that acetate generally inhibits growth of *E. coli* in culture. Rather, the rejection sets forth the express teaching of Shimizu that it is preferable to control the acetate concentration in a culture medium to "6 g/liter or less" (column 4, lines 44-50). Thus, Shimizu prefers to maintain acetate concentration at 6 g/L or less in the culture medium.

Beginning at p. 5, bottom of the Brief, appellant argues that Chang's inability to explain feeble growth of *E. coli* on acetate produced by pyruvate oxidase is an indication of the unpredictable effects of genetic modification. Appellant further argues that Chang does not use L-amino acid production strains and it cannot be concluded that similar results could be achieved using a production strain. According to appellant, only with hindsight reasoning would one combine the reference of Chang with Shimizu.

Appellant's argument is not found persuasive. Chang's inability to explain feeble growth of *E. coli* on acetate produced by pyruvate oxidase (p. 762, column 1) is not critical with respect to whether or not one of ordinary skill in the art would have been motivated to combine Chang with the other references. In other words, an explanation of why *E. coli* exhibits feeble on acetate is not necessary for combining Chang with the other references. Chang acknowledges that pyruvate oxidase (poxB, encoded by *poxB* gene) produces acetate (p. 756, column 1, top) and teaches production of acetate can be blocked by an *E. coli* strain with an inactivated *poxB* gene (p. 762, column 1, top), which is undisputed by appellant. Shimizu teaches that acetate inhibits *E. coli* cell growth at a concentration above 6 g/liter during routine culturing. Thus, one would have been motivated to inactivate a *poxB* gene in the *E. coli* of Kikuchi or Matsui with a reasonable expectation of inactivating its corresponding pyruvate oxidase catalytic activity that results in acetate production to achieve reduced acetate in the culture medium. Although appellant asserts that one cannot conclude that similar results would have been achieved in an *E. coli* of Kikuchi or Matsui, appellant provides no rationale or line of reasoning to support this allegation. To the contrary, since the teachings of Shimizu and Chang are both directed to *E. coli* and since the method of Kikuchi or Matsui uses *E. coli*, one of ordinary skill in the art would have had a reasonable expectation that inactivating the *poxB* gene in the *E. coli* of Kikuchi or Matsui would have had the effect of inactivating its corresponding pyruvate oxidase catalytic activity.

In response to appellant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that

any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the appellant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

Beginning at p. 6, bottom of the Brief, appellant argues that the pathway involving poxB is not the primary source for acetate production, citing to p. 6657, column 2 of the reference of Chang et al. (J. Bacteriol. 181:6656-6663, 1999; copy attached to the Brief) and the overview of the KEGG-Pathways (copy attached to the Brief).

Appellant's argument is not found persuasive. The examiner has reviewed the noted statement in appellant's cited reference of Chang et al. (J. Bacteriol. 181:6656-6663, 1999) and the overview of the KEGG-Pathways, however, this statement does not appear to disclose – either explicitly or implicitly – that "the pathway in which poxB is involved is not the primary source of acetate in a cell" as alleged by appellant. Although the examiner has previously requested clarification as to how appellant has arrived at this conclusion, no further clarification has been provided. In the absence of such clarification, it is unclear to the examiner as to how appellant this conclusion has been reached. The rejection does not assert that pyruvate oxidase is the *only* enzyme that can produce acetate in an *E. coli* or that inactivating the *poxB* gene in *E. coli* will *eliminate* acetate production. Instead, the rejection states that *most* of the endogenous

production of acetate is blocked in an *E. coli* strain with an inactivated *poxB* gene, which is expressly taught by the reference of Chang (p. 762, column 1, top to middle).

Perhaps appellant's intention in citing to the statement of appellant's cited reference of Chang et al. (J. Bacteriol. 181:6656-6663, 1999) is to suggest that one of skill in the art would have inactivated a *pta* gene rather than a *poxB* gene to reduce acetate accumulation in a culture medium. However, appellant's Chang et al. (J. Bacteriol. 181:6656-6663, 1999) reference notes that a *pta* mutant exhibits a "large growth defect" relative to a wild-type *E. coli* (p. 6661, column 2), while the *poxB* mutant of Chang is disclosed as growing at a normal rate on acetate containing medium and attaining a normal cell density (p. 758, column 1, middle). Because Chang explicitly discloses that poxB polypeptide catalyzes formation of acetate (p. 756, column 1, top) and that most endogenous acetate production is blocked in strains with a deletion of a *poxB* gene (p. 762, column 1, top), one would have been motivated to use an *E. coli* with an inactivated *poxB* gene in the method of Kikuchi or Matsui.

At p. 7 of the Brief, appellant argues that it was generally known that acetate may significantly lower yield and productivity in different processes involving microorganisms, citing to the reference of Xu et al. (*Appl. Microbiol. Biotechnol.* 51:564-571, 1999). However, according to appellant, this is not a particular problem of L-amino acid production and one of ordinary skill in the art would not have sought to lower acetate formation and certainly not by inactivating *poxB*.

Appellant's argument is not found persuasive. There is no evidence of record to support appellant's allegation that acetate accumulation in an *E. coli* culture medium is

"not a particular problem of L-amino acid production". According to MPEP 716.01(b).II, "[t]he arguments of counsel cannot take the place of evidence in the record".

Based on the combination of references, one of ordinary skill in the art would have recognized that the culturing times of Kikuchi and Mitsui result in growth-inhibiting levels of acetate in the culture medium. According to Shimizu, longer culturing times result in greater accumulation of acetate, noting that 6 g/L acetate is reached at 10 hours and 17 g/L is reached at 18 hours (column 4, lines 42-44). According to Shimizu, it is preferable to maintain 6 g/L or less of acetate in the culture medium, where acetate concentration reaches 6 g/L at 10 hours of culturing of *E. coli* (column 4, lines 42-50). The method Kikuchi encompasses culturing of *E. coli* for a time of "from 16 to 72 hours" (column 10, lines 44-45) and the method of Matsui encompasses culturing of *E. coli* for a time of 72 hours (column 4, lines 64-65), thus requiring time periods that reach undesirable growth-inhibiting levels of acetate as noted by Shimizu.

At least for these reasons, the method of claims 23, 26, 28, 33, and 42 would have been *prima facie* obvious at the time of the invention.

At p. 7 of the Brief, appellant notes that the obviousness rejections directed to claims 27 and 39-41 are not separately argued. The examiner maintains that at least for the reasons set forth above, the method of claims 27 and 39-41 would have been *prima facie* obvious at the time of the invention.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

David J. Steadman

/David J. Steadman/
Primary Examiner, Art Unit 1656

Conferees:

/Manjunath N. Rao /

Supervisory Patent Examiner, Art Unit 1656

Robert Mondesi

/Robert B Mondesi/

Supervisory Patent Examiner, Art Unit 1652